

A Study on Disinfection Performance of Mobile Airborne Disinfection System with Hydrogen Peroxide in Complex Areas

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Abstract

Airborne Surface Disinfection (ASD) effectively neutralizes surface bioburden using a combination of specialized devices and disinfectants. This crucial technique is widely adopted in sensitive environments like operating theatres, microbiology and virology laboratories, and facilities manufacturing life-saving drugs. Its effectiveness is validated against various microorganisms. The success of ASD depends on several factors: the ASD device, disinfectant type, the target location's complexity and occupancy, and environmental factors such as relative humidity, temperature, and materials present. Hydrogen Peroxide (H₂O₂) is a preferred disinfectant for this application. Complex target areas often necessitate multiple devices or extended disinfection times. Integrating an ASD device onto a robotic platform can significantly enhance efficiency by reducing the need for multiple machines and shortening overall disinfection cycles without compromising efficacy. A study in a 307 cu.mt. complex location investigated the effectiveness of different ASD devices and H₂O₂ concentrations. The study compared a portable device (three units) with MASCA, a fogging device on a robotic platform, using both 6% and 7.5% H₂O₂. Chemical indicators verified disinfectant distribution, while biological indicators confirmed disinfection efficacy, both showing a >4 log reduction in bioburden. Significantly, MASCA with 7.5% H₂O₂ achieved successful disinfection in 124 minutes with a single unit, compared to 181 minutes with three portable devices, saving 58 minutes. Similarly, MASCA with 6% H₂O₂ completed disinfection in 156 minutes versus 197 minutes for the portable units, saving 41 minutes. This demonstrates the superior efficiency of the robotic platform in complex environments.

Keywords: Airborne Surface disinfection (ASD), Hydrogen Peroxide (H₂O₂), Enzyme Indicator (EI), Biological Indicator (BI), Chemical Indicator (CI), Disinfection, Decontamination, MASCA.

1. Introduction

Airborne Surface Disinfection (ASD) is a method that disinfects surfaces using airborne agents like aerosols or mist. This process aims to eliminate or inactivate pathogens such as bacteria, viruses, and fungi on surfaces. It works in conjunction with traditional cleaning, and is especially useful in areas where manual cleaning is difficult or unable to reach all surfaces effectively. The primary components of ASD Typically Include:

Disinfectant Agents: These are chemicals or

substances with antimicrobial properties that kill or neutralize pathogens on contact. Each disinfectant requires a specific contact time to be effective against particular pathogens (Rutala, 2008) (McAnoy, 2006). Hydrogen Peroxide (H₂O₂) is often favoured because it's effective against a wide variety of pathogens and doesn't leave any residue. (Jones, Drake, & Eagleson, 1993).

Aerosol or Mist Generation: The disinfectant agent is converted into fine particles in the form of aerosols

or mists, which can remain suspended in the air for a period, allowing them to reach (Kimball, 2014) and treat surfaces that may be difficult to access with conventional cleaning methods.

Delivery Mechanism: The aerosols or mist are delivered using specialized devices like foggers, sprayers, or diffusers, designed to disperse the disinfectant evenly throughout the target area. (Jones, Drake, & Eagleson, 1993) (Lucchini, 2018) Airborne surface disinfection is valuable in many places, including hospitals, labs, food processing plants, and public transport, especially for large or hard-to-reach areas. However, it's crucial to use the correct disinfectants and follow guidelines to ensure safety and effectiveness. The best disinfectant and method depend on the specific pathogens you're targeting and the surfaces in the area. Always take proper safety precautions for people during disinfection, as some disinfectants can be harmful if inhaled or if they contact skin or eyes. Irrespective of aerosol generation and delivery mechanism, sequence of activity remains the same. (McAnoy, 2006) (Richard, 2023) (Sigwarth & Moirandat, 2000) Pre-Conditioning → Fogging / Gassing Time → Contact Time/HOLD Time → Post-Conditioning Pre-Conditioning Clean rooms typically maintain a relative humidity (RH) between 45% and 65% to prevent condensation, mold, and damage to sensitive equipment. While pre-conditioning RH might be needed for some fogging disinfection methods, it's not universally required. However, maintaining controlled RH in clean rooms is always crucial for proper operation and contamination prevention. Before disinfection, remember to turn off all ventilation systems and seal ducts and doors with duct tape. (Steris Life Sciences, 2000). Fogging or gassing is the phase in the surface disinfection process where the desired concentration of the disinfectant is introduced into the target area in the form of fine aerosol droplets or mist (fog). The purpose of this phase is to distribute the disinfectant throughout the air and onto surfaces, allowing it to come into contact with and eliminate harmful microorganisms. The time required for the fogging or gassing phase can vary depending on several factors, including (Kimball, 2014) (Sigwarth & Moirandat,

2000):

- **Desired Concentration:** The concentration of the disinfectant required to achieve effective (Jones, Drake, & Eagleson, 1993) disinfection will influence the fogging time. Higher concentrations might require longer fogging periods to ensure sufficient coverage and contact with pathogens. (Linley, Denyer, McDonnell, Simons, & Maillard, 2012) (Klapes NA, 1990)
- **Room Size:** The larger the target area, the more time it may take to evenly disperse the disinfectant fog throughout the space. Larger rooms might require additional time to ensure comprehensive coverage. (Steris Life Sciences, 2000)
- **Room Complexity:** The layout and complexity of the room can also affect the distribution of the fog. Rooms with numerous obstructions, intricate structures, or challenging airflows may need more time to ensure that the fog reaches all surfaces and corners. (NET, 2018) (Krishnan, Berry, Fey, & Wagener, 2006)
- **Type of Fogging Equipment:** The type and efficiency of the fogging or gassing equipment used can impact the speed and effectiveness of the process. Advanced fogging systems may be able to achieve the desired concentration more quickly and uniformly. (Ku'min, Albert, Weber, & Summermatter, 2020)
- **Disinfectant Characteristics:** Different disinfectants have varying properties, such as their evaporation rate and the time needed to remain in contact with pathogens to be effective. These characteristics can influence the fogging time.

Contact time also known as hold time or dwell time, is a critical aspect of the surface disinfection process (Mørretrø, Fanebust, Fagerlund, & Langsrud, 2019) (Ku'min, Albert, Weber, & Summermatter, 2020). It refers to the duration during which the disinfectant needs to remain in contact with the surfaces or air to effectively deactivate or kill biological agents like bacteria, viruses, and other pathogens. The

importance of contact time lies in its role in achieving thorough disinfection. When the disinfectant is in contact with the target surfaces or suspended in the air for the appropriate duration, it allows the active ingredients in the disinfectant to interact with and destroy the microorganisms effectively (Saini, Sikri, Batra, Kalra, & Gautam, 2020). If the contact time is too short, the disinfectant may not have sufficient time to work, potentially leaving behind viable pathogens and reducing the overall effectiveness of the disinfection process. The required contact time can vary depending on the type of disinfectant used, the specific pathogens targeted, and the device and disinfectant manufacturer's recommendations (Lee GH, 2023). Different disinfectants have distinct kill times for various microorganisms. In addition to killing the biological agents, the contact time also contributes to achieving homogeneity in the distribution of the disinfectant in the area. Adequate contact time allows the disinfectant to reach all surfaces, including hard-to-reach areas, ensuring comprehensive disinfection. Post-conditioning is the final phase in the surface disinfection or decontamination process, and it involves several important steps:

- **Removal of Residual Chemical:** After the contact time (or hold time) has elapsed, the residual disinfectant or chemical left on surfaces or in the air needs to be removed. This step is crucial to ensure the area is safe for occupants to re-enter and to prevent any potential adverse health effects from prolonged exposure to the disinfectant. [1]
- **Regulation of Relative Humidity (RH):** During the fogging or gassing phase, the relative humidity in the target area might have been increased to aid in the effectiveness of the disinfection process. In the post-conditioning phase, the relative humidity is often restored to the desired level for the particular environment, such as clean rooms maintained at 45% - 65% RH.
- **Restoration of Air Pressure Balance:** In cases where multiple rooms or areas are decontaminated simultaneously, it is essential to restore the air pressure balance between

these spaces. This ensures that air can flow appropriately, maintaining a controlled environment and preventing cross-contamination between different areas.

Post-conditioning is a critical part of the disinfection process to ensure the area is safe for re-entry and to maintain the desired environmental conditions for the specific application. Proper post-conditioning helps to mitigate any potential risks associated with the disinfection procedure and prepares the space for normal use after decontamination. It is essential to follow recommended procedures and safety guidelines during post-conditioning to achieve effective disinfection while considering the well-being of individuals and the integrity of the environment. (Ku'min, Albert, Weber, & Summermatter, 2020) These sequence of events demands a cycle time of 4 – 12 hours depending on the device and disinfectant combination (Jones, Drake, & Eagleson, 1993). This total downtime can be reducing by reducing the “Fogging Time” and/or “Contact Time”. The total downtime required for the entire disinfection process can be reduced by optimizing the fogging time and contact time. [2]

- **Fogging Time:** As mentioned earlier, fogging is the phase where the disinfectant is dispersed as fine aerosol droplets or mist throughout the target area. The fogging time can be reduced by using more efficient fogging equipment or systems that disperse the disinfectant rapidly and evenly. Fogging equipment when integrated with a mobile platform (Andersen BM, 2006) would be doing this job efficiently covering larger areas in less time, thus reducing the overall downtime. [3]
- **Contact Time:** The contact time, also known as hold time or dwell time, is the duration the disinfectant needs to remain in contact with the surfaces or air to effectively kill the pathogens (Lee GH, 2023) (Gamage, 2003). While it is essential to follow the manufacturer's recommendations to ensure proper disinfection, some disinfectants may have shorter contact times for certain pathogens. Using disinfectants with shorter

contact times, where appropriate and effective, can help reduce the overall downtime. Additionally, if the fogging device is able to retain the disinfectant concentration for longer times in the target area, contact time could be reduced. By optimizing these two factors, it is possible to minimize the downtime required for the surface disinfection or decontamination process while still ensuring the effectiveness of the procedure. Effectiveness of this combination would be verified by exposing the Chemical, Biological and Enzyme Indicators, Relative Humidity would also be monitors as it is one of the critical parameters that influences effectiveness. [4]

- Chemical Indicator or semi-quantitative strips that were used in this case would change colour when exposed to the chemical. (MACHEREY-NAGEL, n.d.) [5]
- Biological indicators play a crucial role in verifying the effectiveness of H₂O₂ decontamination processes. Biological indicators used for this are typically consist of highly resistant spores of bacteria, such as *Geobacillus stearothermophilus* (formerly *Bacillus stearothermophilus*) (Murphey, 2007). These spores are chosen because of their ability to withstand harsh conditions and their similarity to the resistance of other microorganisms, including more resistant viruses [6]

Enzyme indicators, also known as enzymatic indicators, are an alternative approach to using traditional biological indicators like spores for verifying the effectiveness of H₂O₂ decontamination processes (McLeod NP, 2017) (Schachtschneider A, 2022). Enzyme indicators utilize enzymes that are sensitive to the decontamination conditions, and their activity is measured before and after the decontamination to determine the success of the process. These indicators offer both quantitative and qualitative results, closely correlated to biological indicators. The Enzyme Indicator is a strip containing a specific quantity of enzyme that degrades upon exposure to the oxidization process. To analyse the

exposed indicators, a luminescent assay is applied, and readings are interpreted using a photo-multiplier manufactured by Berthold Technologies, GMBH, along with Athena software. Protak Scientific, UK, is the manufacturer of the Enzyme Indicators, luminescent assay, and Athena software. [7]

2. Methods

MASCA, or "Mobile Airborne bio-decontamination System for Cleanroom Asepsis," is a custom-designed Airborne Surface Disinfection (ASD) device. It integrates a robotic platform to navigate a pre-defined path, while an ultrasonic nebulizer converts 7.5% Hydrogen Peroxide (H₂O₂) into a fine mist for fogging. This experimental study aimed to determine if a single MASCA device with 7.5% H₂O₂ could effectively replace multiple ASD devices in moderately complex locations and identify the optimal disinfection time. The study utilized chemical, biological, and enzyme indicators to assess the bio-decontamination process. All indicators were expected to pass in both operational modes, with chemical indicator results helping to understand the diffusion pattern. The target location was a laboratory testing area, a controlled environment with relative humidity maintained between 45%–65% and temperature between 24°C–27°C by an automated air handling system. This slightly complex area, consisting of six rooms and two corridors, totalled 307 cubic meters. Due to its layout and occupancy, three stationary ASD devices would typically be required. [8-10]

The study compared two ASD systems:

- Portable ASD device: A handy, portable system with a diffusion rate of 20 ml/min, generating droplets around 4-5 µm.
- MASCA system: An airborne disinfection system integrated with a programmable robotic platform. It uses ultrasonic nebulizers to create mist from liquid biocide, capable of generating 4 µm droplets and a maximum diffusion rate of 40 ml/min. For this study, a diffusion rate of 30 ml/min was used.
- The disinfectant used was 6% H₂O₂ and 7.5% H₂O₂ from VM Sciences, India's HyPer™ range, without added stabilizing agents or impurities. [11]

- **Chemical Indicators** The semi-quantitative strips used in this study were manufactured by MACHEREY-NAGEL GmbH, a product reference number of 91319. Before the decontamination activity, strips were placed in various designated locations within the target area. After the decontamination process, these strips were carefully collected and the colour change was observed. If the colour changed from white to blue, it indicated that H₂O₂ had reached the location of the indicator strip. (Richard, 2023) [12]
- **Biological Indicators** used in this study is commercially available as ready to use indicator manufactured by True Indicating LLC, USA for monitoring vaporised hydrogen peroxide decontamination. A population of 2.4×10^4 of *Geobacillus stearothermophilus* (ATCC 12980) is inoculated per 6 mm stainless steel disc. Lot no S15-4 with expiry date of 08th Jan 2023 is used. D-Value is of 1.7min (Murphey, 2007). Placed 13no in all designated locations in the target location before the decontamination activity. After exposure of the indicator to Hydrogen Peroxide vapor, the indicators are collected and aseptically transferred each disc to individual tube containing 15-20ml of Soybean Casein Digest Broth (SCDB), incubated for 7 days at 55°-65°C, a positive control is also maintained for each run. No growth is observed in the exposed indicators implying bio-decontamination is effective. In case of failure cream coloured sediment growth would be observed. (Richard, 2023)
- **Enzyme Indicators** offer an alternative to biological indicators, providing a rapid microbial assessment specifically designed for hydrogen peroxide-based decontamination systems. During the decontamination process, indicators are strategically placed in various corners and designated locations within the target area. Once exposed, these indicators are carefully transported to the laboratory for analysis. One of the key advantages of using enzyme

indicators is the immediate result they provide, eliminating the need to wait for a seven-day incubation period. Furthermore, the results obtained are not binary; they offer both quantified and qualified outcomes, providing more comprehensive information. (Richard, 2023) [13]

2.1. Experimental Setup

An experimental study was conducted in a complex location comprising six rooms and two connecting corridors. This area, previously determined to require three standard ASD devices (each with a 150 cu. mt. operational capacity and 20 ml/min diffusion rate) for decontamination, presents a challenge due to the limited "throw distance" of vapor/mist generated by individual devices. To overcome this, either larger capacity devices or multiple units are typically needed. In this study, a single MASCA device, utilizing its mobile platform, was employed. To validate its effectiveness, 13 chemicals, biological, and enzyme indicators were strategically placed at equidistant points throughout the area. The chemical and device combination was specifically validated for achieving a 4-log reduction of *Geobacillus stearothermophilus* bacteriological spores. Effective decontamination is about maintaining desired concentration (as per the D-Value (Sigwarth & Moirandat, 2000) of the target organism) for a desired time. Manufacturers of commercial indicators provide the same information along with the certificate of analysis. The important modification made here is usage of only one MASCA device and no contact time (120 mins). Total experimental study is done in 4 stages: [14]

The study involved four distinct stages to compare the performance of portable ASD devices with the MASCA system under different conditions:

Stage 1: Portable ASD with 6% H₂O₂

Three portable ASD devices, each with a diffusion rate of 20 ml/min, were strategically placed as shown in Figure 1. Using a 6% H₂O₂ concentration at a dosage of 15 ml/cu.mt, the total cycle time was 197 minutes, which included 77 minutes of fogging and a 120-minute contact time. Approximately 4600 ml of disinfectant was diffused. [15]

Stage 2: MASCA with 6% H₂O₂

The MASCA device, operating at a diffusion rate of 30 ml/min, followed a pre-defined path from point A to point O and back (a 52-meter distance covered in about 6.5 minutes), as illustrated in Figure 1. With the same 6% H₂O₂ concentration and 15 ml/cu.mt dosage, MASCA completed the full cycle in 156 minutes, diffusing roughly 4600 ml of disinfectant.

Stage 3: Portable ASD with 7.5% H₂O₂

Similar to Stage 1, portable ASD devices were positioned as shown in Figure 1. This stage used a 7.5% H₂O₂ concentration at a lower dosage of 12 ml/cu.mt and a diffusion rate of 20 ml/min. The total cycle time was 181 minutes (61 minutes of fogging plus 120 minutes of contact time), diffusing approximately 3680 ml of disinfectant. [16-17]

Stage 4: MASCA with 7.5% H₂O₂

In this final stage, MASCA followed the same A-O-A path as in Stage 2, covering 52 meters in about 6.5 minutes. Utilizing 7.5% H₂O₂ at a 12 ml/cu.mt dosage and a 30 ml/min diffusion rate, MASCA achieved full disinfection in just 124 minutes, diffusing approximately 3680 ml of disinfectant. (Figure 1)

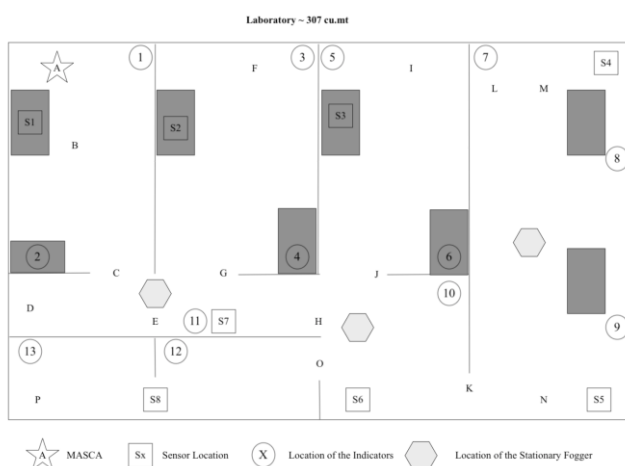


Figure 1 Experimental Setup

3. Results & Discussion

This experimental study was conducted in a single location, but across four stages, each employing different chemical and device combinations. Tables I through IV summarize the indicator results for each day. For chemical indicators, a "+" symbol signifies a successful outcome, meaning the indicator's color changed from white to blue upon exposure to the

disinfectant vapor or mist. All chemical indicators in this study passed. Regarding biological indicators, a "+" symbol denotes failure, indicating observed growth after the incubation period. Conversely, a "-" symbol signifies success, meaning no growth was observed and the indicator was deactivated by the decontamination. All biological indicators in this study also passed. Enzyme indicators provided quantified values of log reduction levels for *Geobacillus stearothermophilus* (ATCC 12980) in Relative Light Units (RLU), with a multiplication factor applied. All tested locations consistently demonstrated a high level of log reduction, specifically greater than a 4-log reduction. (Figure 1,2,3) [18-19]

Table I Results of all Indicators - Experimental Setup - 1

ID	Chemical Indicator			Biological Indicator			Enzyme Indicators		
	Day-1	Day-2	Day-3	Day-1	Day-2	Day-3	Day-1	Day-2	Day-3
1	+	+	+	-	-	-	4.3	4.2	4.2
2	+	+	+	-	-	-	5.8	5.9	5.6
3	+	+	+	-	-	-	4.4	4.1	4.0
4	+	+	+	-	-	-	5.5	5.5	5.5
5	+	+	+	-	-	-	4.0	4.0	4.1
6	+	+	+	-	-	-	4.7	4.3	4.6
7	+	+	+	-	-	-	4.3	4.3	4.9
8	+	+	+	-	-	-	5.4	5.6	5.3
9	+	+	+	-	-	-	4.5	4.5	4.0
10	+	+	+	-	-	-	6.9	6.8	7.1
11	+	+	+	-	-	-	7.2	7.3	8.8
12	+	+	+	-	-	-	4.4	4.5	4.2
13	+	+	+	-	-	-	4.0	4.0	4.1

Figure 2 Stage 1

Table II Results of all Indicators - Experimental Setup - 2

ID	Chemical Indicator			Biological Indicator			Enzyme Indicators		
	Day-1	Day-2	Day-3	Day-1	Day-2	Day-3	Day-1	Day-2	Day-3
1	+	+	+	-	-	-	4.3	4.3	4.1
2	+	+	+	-	-	-	4.4	4.3	4.1
3	+	+	+	-	-	-	4.3	4.3	4.4
4	+	+	+	-	-	-	4.4	4.5	4.5
5	+	+	+	-	-	-	4.4	4.2	4.1
6	+	+	+	-	-	-	4.2	4.3	4.2
7	+	+	+	-	-	-	4.5	4.4	4.4
8	+	+	+	-	-	-	4.1	4.0	4.0
9	+	+	+	-	-	-	4.1	4.2	4.1
10	+	+	+	-	-	-	4.3	4.4	4.3
11	+	+	+	-	-	-	4.5	4.4	4.4
12	+	+	+	-	-	-	4.3	4.2	4.4
13	+	+	+	-	-	-	4.4	4.2	4.2

Figure 3 Stage 2

Table III Results of all Indicators - Experimental Setup - 3									
ID	Chemical Indicator			Biological Indicator			Enzyme Indicators		
	Day-1	Day-2	Day-3	Day-1	Day-2	Day-3	Day-1	Day-2	Day-3
1	+	+	+	-	-	-	4.7	4.6	4.6
2	+	+	+	-	-	-	6.2	6.3	6.3
3	+	+	+	-	-	-	4.4	4.5	4.1
4	+	+	+	-	-	-	5.8	6.5	6.2
5	+	+	+	-	-	-	4.1	4.0	4.1
6	+	+	+	-	-	-	5.1	5.5	5.3
7	+	+	+	-	-	-	4.5	4.5	4.5
8	+	+	+	-	-	-	6.1	6.1	6.4
9	+	+	+	-	-	-	4.6	4.5	4.5
10	+	+	+	-	-	-	7.3	7.3	7.1
11	+	+	+	-	-	-	7.9	8.4	7.5
12	+	+	+	-	-	-	5.3	5.2	5.2
13	+	+	+	-	-	-	4.0	4.2	4.1

Figure 4 Stage 3

Table IV Results of all Indicators - Experimental Setup - 4									
ID	Chemical Indicator			Biological Indicator			Enzyme Indicators		
	Day-1	Day-2	Day-3	Day-1	Day-2	Day-3	Day-1	Day-2	Day-3
1	+	+	+	-	-	-	4.8	4.7	4.7
2	+	+	+	-	-	-	4.5	4.4	4.5
3	+	+	+	-	-	-	4.7	4.6	4.5
4	+	+	+	-	-	-	4.4	4.5	4.5
5	+	+	+	-	-	-	4.4	4.4	4.4
6	+	+	+	-	-	-	4.5	4.1	4.6
7	+	+	+	-	-	-	5.0	4.9	4.5
8	+	+	+	-	-	-	4.3	4.3	4.3
9	+	+	+	-	-	-	4.3	4.5	4.1
10	+	+	+	-	-	-	4.7	5.2	5.5
11	+	+	+	-	-	-	6.0	5.9	6.2
12	+	+	+	-	-	-	4.9	4.8	5.3
13	+	+	+	-	-	-	4.8	4.8	5.5

Figure 5 Stage 4

Conclusion

- One ASD device integrated on mobile platform is sufficient to decontaminate a complex location of up to 300 cu.mt instead of 3no of stationary ASD units.
- Contact time can be completely avoided if the fogging time is maintained for a period of 120min or more, ASD device to be in mobile mode.
- When using MASCA, using 7.5% H₂O₂ is also reducing the overall cycle time.
- Overall capital investment, running cost can be reduced drastically. [20-22]

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